

Figure 1A

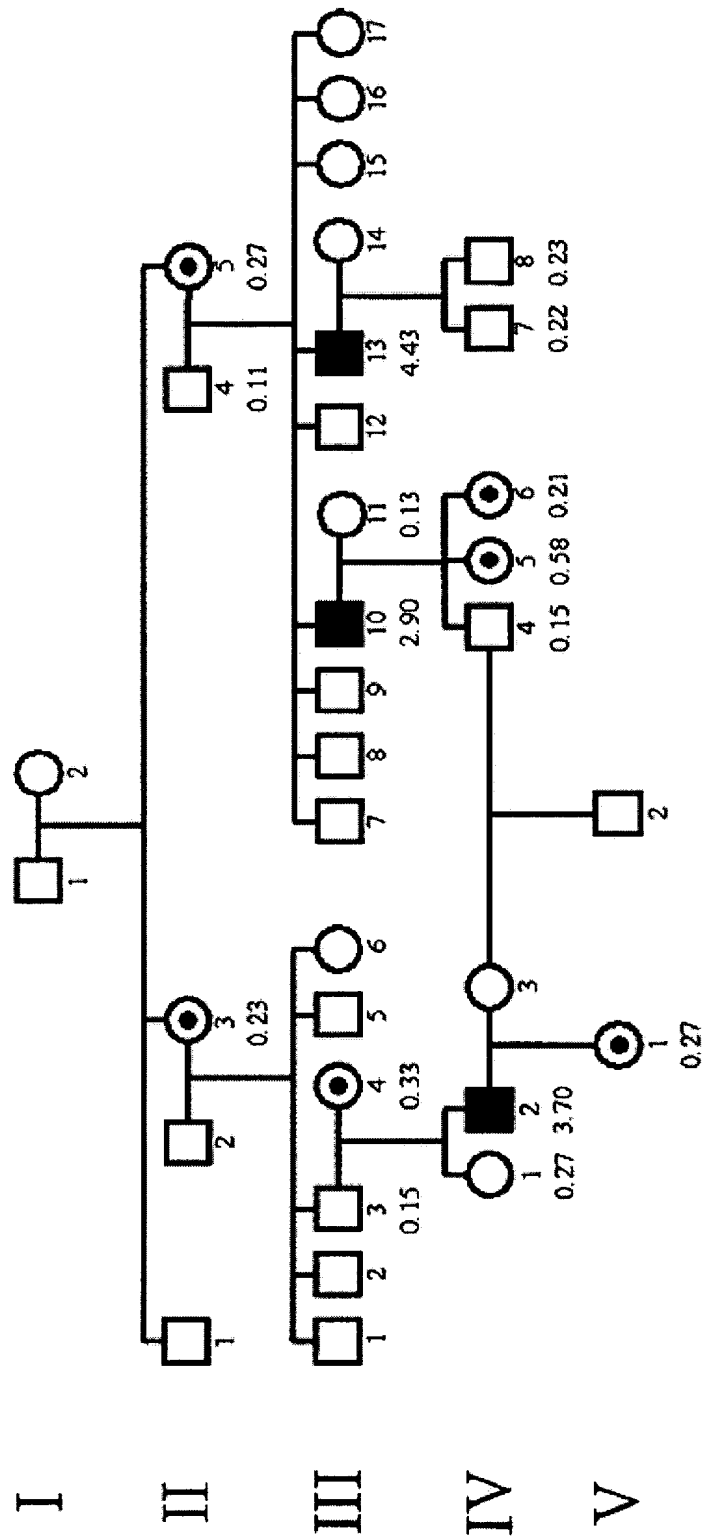


Figure 1B

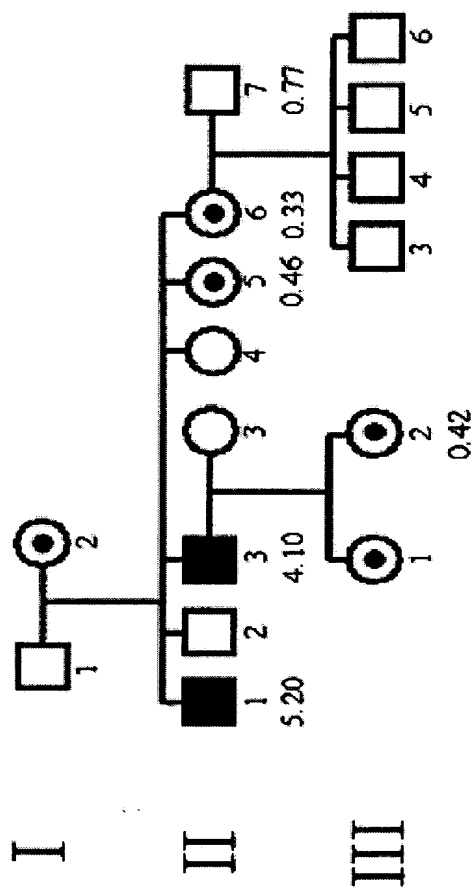
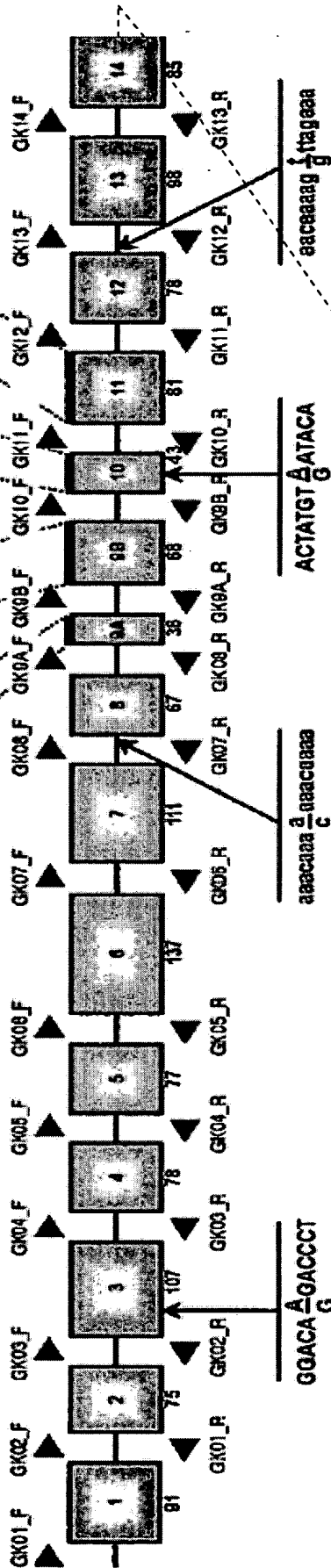


Figure 1C



ag:AA...GG:gt ag:TG...AC:gt ag:GT...AG:gt ag:TG...AA:gt

100 bp



931 C 24

1150 E 8

ag:AA...TG:gt

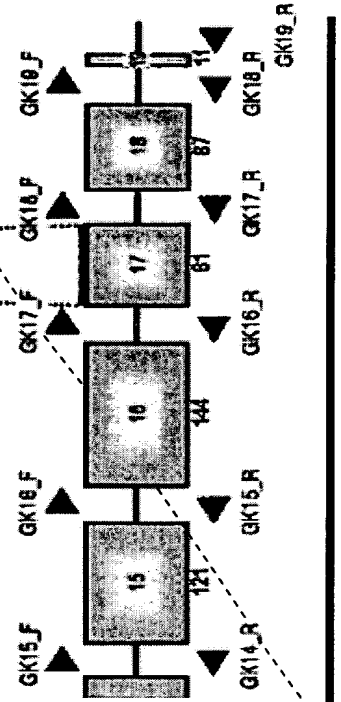


Figure 2



Figure 3A

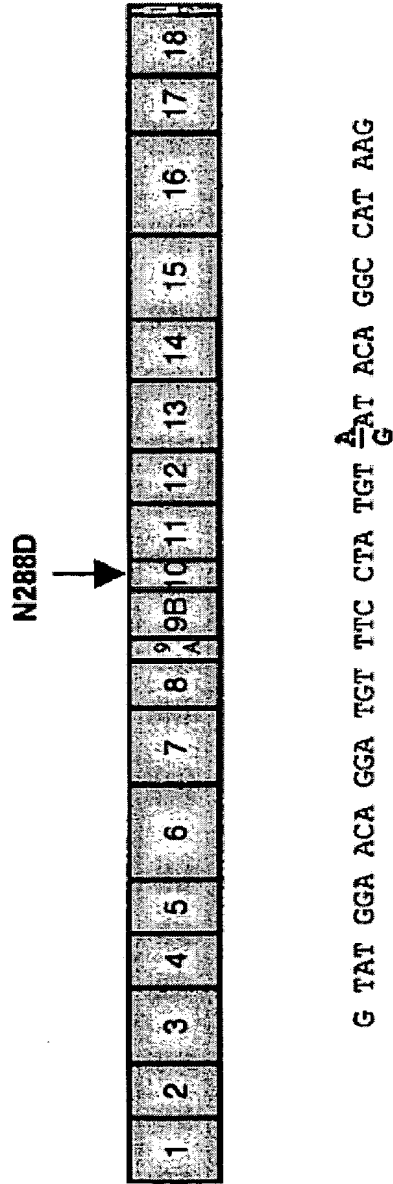


Figure 3B

270		310	
GK N288D Mutant		SEQ ID NO: 6	
glpk_human	FOIGQAKNTYGTGCFLLDNTGCHKCVFSDHGLLTTVAYKLGR	SEQ ID NO: 7	
glpk_rat	FOIGQAKNTYGTGCFLLDNTGCHKCVFSDHGLLTTVAYKLGR	SEQ ID NO: 8	
glpk_mouse	FQDGGAKNTYGTGCFLLDNTGCHKCVFSEHGLLTTVAYKLGR	SEQ ID NO: 9	
glpk_ecoli	VKEGMAKNTYGTGCFMLMNTGEKAVKSENGLLTTIAC--GP	SEQ ID NO: 10	
glpk_pseae	VEPGQAKNTYGTGCFLLDNTGDKAVKSTHGLLTTIAC--GP	SEQ ID NO: 11	
glpk_entca	FEKGMKNTYGTGAFIVMTGEEPQLSDNDLLTTIGY--GI	SEQ ID NO: 12	
glpk_haein	VHAGQAKNTYGTGCFMLLHTGNKAITSKNGLLTTIACNAKG	SEQ ID NO: 13	
glpk_bacsu	FEEGMGKNTYGTGCFMLMNTGEKAIKSEHGLLTTIAW--GI	SEQ ID NO: 14	
glpk_yeast	YKPGAARCTYGTGCFLLDNTGTTKKLISQHGALTTLAFWFFH	SEQ ID NO: 15	
glpk_myoge	TEPGMVKNTYGTGCFVLMNIGDKPTLSKHNLLTTVAWQLEN	SEQ ID NO: 16	
glpk_entfa	FERGMVKNTYGTGSFIVMTGEEFPQLSKNNLLTTIGY--GI	SEQ ID NO: 17	
glpk_myopn	VEPAMVKNTYGTGCFMLMNI GNELKYSQHNLLTTVAWQLEN	SEQ ID NO: 18	
glpk_syny3	DRPGLLKCTYGTGAFIVANTGQTVTRSQHRLLSLTVAWTQTN	SEQ ID NO: 19	

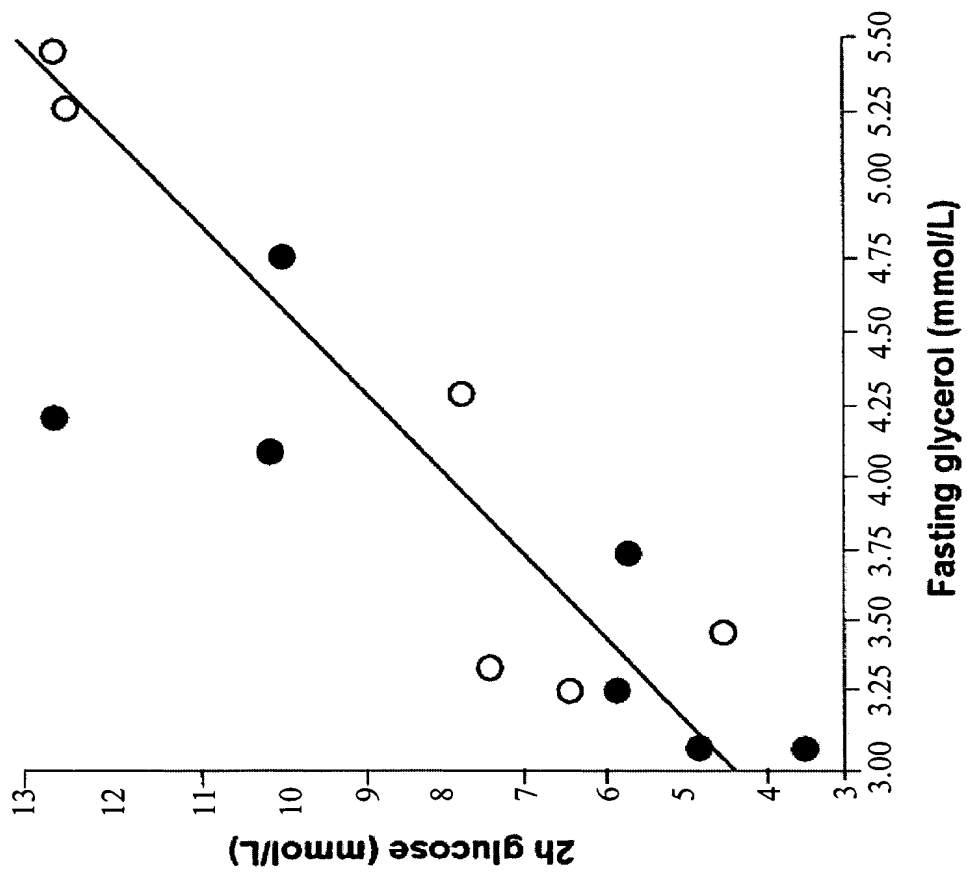


Figure 4A

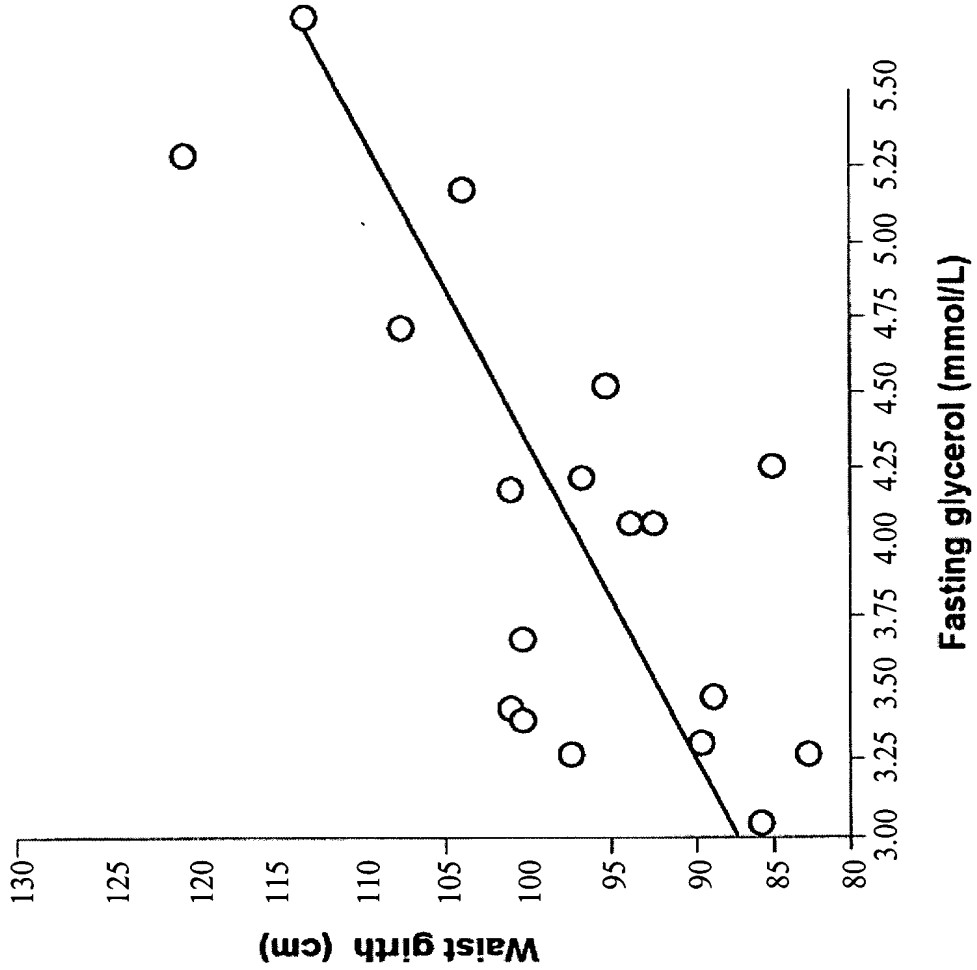


Figure 4B

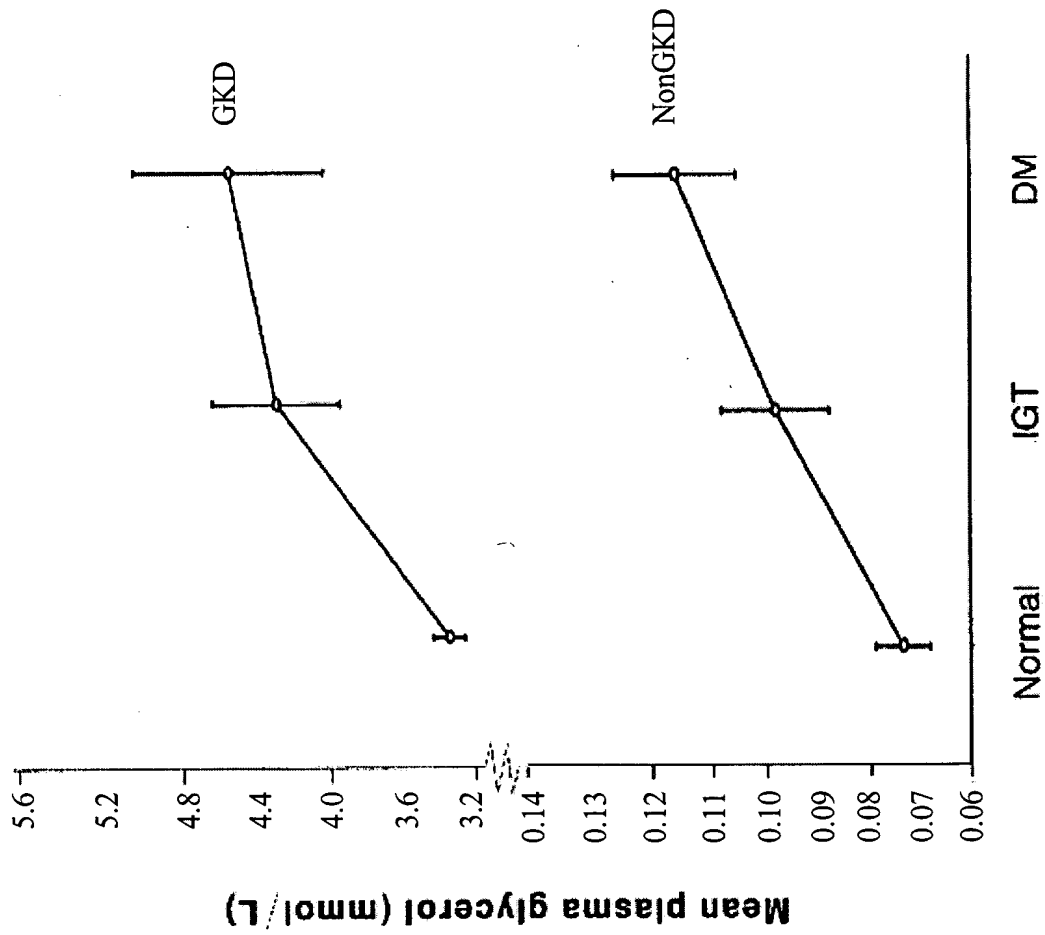


Figure 4C

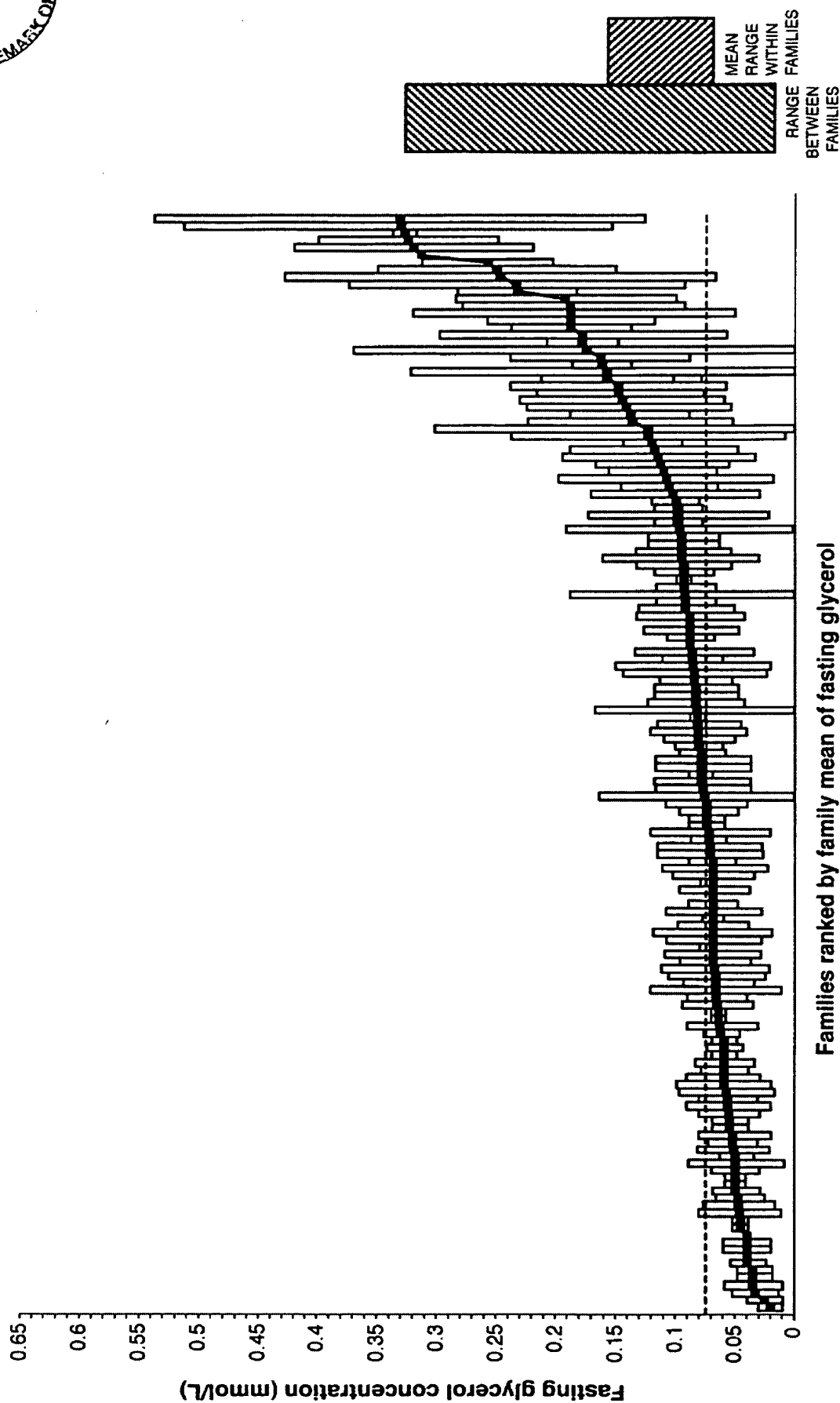
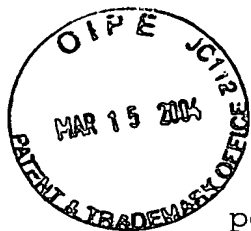


Figure 5



Application No. 09/694,088
Title: GLYCEROL AS A PREDICTOR...
Inventors: Daniel Gaudet, *et al.*

poly: A/G
location: 13th base of exon 3

ATGCCTTCTTTTGTCAAAGATGGGTGGAACA [A/G] GACCCTAAGGAAATTCTACAT
TCTGTCT **SEQ ID NO: 1**

CAA vs CAG ==> silent

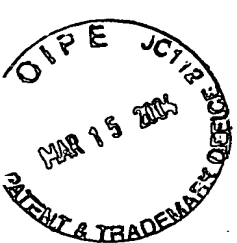
poly: A/C
location: 17th base of intron 8
TAATGGTAAAAACAAACAAA [A/C] AAACAAAAACACACCAAAAAACCAA
 SEQ ID NO: 2

poly: A/G
location: 29th base of exon 10
TTCATTCTCCCTTCAACCATAGGTATGGAACAGGATGTTTCTTACTATGT [A/G] AT
ACAGGCCATAAGGTtGGTTTTTAATAAAAATGATTAAGTCA **SEQ ID NO: 3**

AAT vs GAT ==> N to D

poly: G/T
location: 22nd base of intron 12
GAAATTGGTGAGTGTGTTCTAACAAAAG [G/T] TTAGAAAATCTGAAAATGACACA
TTTCA **SEQ ID NO: 4**

Figure 6



SEQ ID NO: 5

Exon 1:

GGTTCAGCGGACGCGCGCGGCCTCGGTCTCTGGACTCGTCACCTGCCCCCTCCCCCTCCCGCC
GCCGTCACCCAGGAAACCGGCCGCAATCGCCGGCCGACCTGAAGCTGGTTTCATGGCAGCCT
CAAAGAAGGCAGTTTTTGGGGCCATTGGTGGGGGCGGTGGACCAGGGCACCAGTTCGACGCGC
TTTTTGGTGAGCCCGGGGTGACATGTGAAGAGGCGCTGAGC

Exon 2:

TGTAAACGACGGCCAGTCATCCTTGATATCTGCCTGCATTTTTTACATTAATATTACAATAT
CTTTTTCAGGTTTTCAATTCAAAAACAGCTGAACTACTTAGTCATCATCAAGTAGAAATAAA
ACAAGAGTTCCCAAGAGAAGGGTATGTTTCCTAATTTAATATGTAAAGACACATTATGTTTG
TTAGTCCATCTCACCCAACCTGCCC

Exon 3:

CAATGCCTTCTTTTGTCAAAGATGGGTGGAACA [A/G] GACCCTAAGGAAATTCTACATTCT
GTCTATGAGTGTATAGAGAAAACATGTGAGAAACTTGACAGCTCAATATTGATATTTCCAA
CATAAAGGTATTTTAGTAGAATATTTTACCCACA

Exon 4:

TGTAAACGACGGCCAGTTGAGAGCTGTTTTCTGAAGTAGTTCCTACTTGTTAAATTTTTG
ACTTCCTTCTGTTTAACTTTCTCTTTAAAGCTATTGGTGTGAGCAACCAGAGGGAAACCACT
GTAGTCTGGGACAAGATAACTGGAGAGCCTCTCTACAATGCTGTGGGTAAAGCTGTCATGCAT
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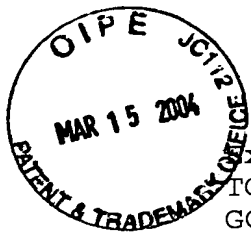
Exon 5:

TGTAAACGACGGCCAGTTCCTTGATAGTGATTTTCAGTAAGTTCCTATTTTTTTTAAATGAAG
TTTTTCATGTATATTATTTTATTTTGGTCTATAGTGTGGCTTGATCTAAGAACCAGTCTAC
CGTTGAGAGTCTTAGTAAAAGAATTCCAGGAAATAATACTTTGTCAAGGTAAGAATTTCTT
CAGAAGTATACTATAAGAATGTTTCTTTTTTTAAAAAAGTTTGCAGATTTCACTAGAAAGA
AGCATCTTATGGTACAATAGTTATTTGATACAATTTATAGAATCTTTTTTCCCGGATAATTGA
GGCC

Exon 6:

TGTAAACGACGGCCAGTTTCTTTTGTGTTGGTGGTTTGTGTTTAACTGTTACACTTTTCAT
TTGCTAACTGAACTTCACAACTGCTTTTAGTCCAAGACAGGCCTTCCACTTAGCACTTACTT
CAGTGCAGTGAACTTCGTTGGCTCCTTGACAATGTGAGAAAAGTTCAAAAGGCCGTTGAAG
AAAAACGAGCTCTTTTTGGGACTATTGATTCATGGCTTATTTGGGTATGTTTAAATATAATG
GATATATGGAGAATTTTTTCAGAAATTTTTCTAGACTGCCTTGCTTATTGTTTCTACTAGC
AGGTCAGACTTTTTAATTAGCA

Figure 7A



Exon 7:

TGTA AACGACGGCCAGTTGTGCTCTGCTGATTATGACCCTTAACAATATGTAAATTAAATT
GCCAATAAGTACAAATTTAACCTGATTTTTTTTACTCTGCCTAGAGTTTGACAGGAGGAGTCA
ATGGAGGTGTCCACTGTACAGATGTAACAAATGCAAGTAGGACTATGCTTTTCAACATTCAT
TCTTTGGAATGGGATAAACAACTCTGCGAGTAAGTTCTGTTTTGCTCTAAATATAGTTTTCC
CAATACACTACCTATTTATAACCGAAATCTTAATATTTTCAGATGTCAGTGGAGCA

Exon 8:

TGTA AACGACGGCCAGTACAGTGTTAAATACCCAATCTTCTTGTTTTTCAGATTTTTTTGGA
ATTCCAATGGAAATTCTTCCAAATGTCCGGAGTTCTTCTGAGATCTATGGCCTAATGGTAAA
AAACAAACAAA [A/C] AAACAAAAACACACCAAAAAACCAAAAAACAAACAAAAAAAACC
TAATAATTAAAGTTTTTTTTTATTACAAAACAAGTTTACTATTCATAATTCAAAGTCAACTGT
GTTATGTTTTGTGACTTAAAACTTTACAGTCCTTTTTTACAATGG

Exons 9A and 9B

AAAGCTGGGGCCTTGGAAGGTGTGCCAATATCTGGGGTAAGTTTCATCACCAAGTGTCTCCC
CATCCCCACCCTTCCCCATGTTATGGCTTTCCTCCTCTTAGTTTCATCAGTGTGCCTCTTTTT
AACTAGGGAAAACAAGTAAAAGTTGCAAAATTGGANNNTCTTGTTCTTACATGTCATACT
GTGGGCCATTGAGAATCTTTTGAATAAATTAATTTTAACTCTCCCTTCCCATACCTATTATC
TTACATATTAACAAATGGTATTAACAAATGGGGAAAATGGCCAAATGGAGAAAATGCAAGGA
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GTGAGTTTAAGAAACAGACTTAAAAACCAATGCTGTTTTGTTTTTTCTACTTGGTGCTTTGA
ATAAGGAAAAGCTTTTGAAGTTCATCCAGGATGAAAATCAATAGCTTAATAGCTCCAATATG
CATATATACACTTTTTTACCATTTTTTTTATATCTTTAAATAAAATACAAA
TGCCATATATATGCACACTGATGAAGCTTATAAAGACCTAAATTTGTAGGCTGGGCGCGG

Exons 10 and 11:

TTATTTGCTTTCAATAAAATTGTCTTCTATTTCATTCTCCCTTCAACCATAGGTATGGAACAG
GATGTTTCTTACTATGT [A/G] ATACAGGCCATAAGGTTGGTTTTTTTAAATTAAAAAATTGA
TTTAAAGTCTAAGTTCATCTAAATAATGCTTGAACATAATTTACTATTAAACAACCTTTTAG
TCTTTAGCTTTTACTTAATCTTTATCAGGGTTTAATTTAGAGCTCAATACAAAATTTGAATC
GTTCTAATAAGAACCATTTTAGACTCTTTGAATTTTATATGTGTGTTTTTAATTGTGCTGGG
GGGAAATCTAGACTGAGACCTCATCAAATCTTAATGCAATCTAATTTGAAACAAGGAATA
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TAAAAATTGGTTTATTGATTGCATTATTTTGTACCTATGTTATTTTAACTTTAAAAAAAAGT
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ATTTTCTGATCATGGCCTTCTCACCACAGTGGCTTACAACTTGGCAGAGACAAACCAGTAT
ATTATGCTTTGGAAGTAAGTTCTTTTTTAATCAATATGGATAATATGACAAACATTCAAAGCT
AATAAAAATCACAGAGTTTCTAACACTTTTCTGGTAAATCTTAATACAGAGGACTCAAAAA
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TCACAGGAGACCTTGATTAGATTGGTTCCTCAGTTCTTATGCCAATTAATCATGTACCTTA
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CGGATTAACGTGCTCTCTAACATTTCTTTCATCTTGAAAATCTTTGATTTTATAAATAAAA
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TCCTCATAAAAAATAAATTTTGTGTAACATTTTGATATAGAAAAGAAAGCGACGAGATTTATG
CCACTTATCACTGGAAACATTTGTTTCAAACATTTTTTGTATGTTATAGTAGGAATATGCCAG
CCTAAGCCTATA

Figure 7B



Exon 12:

TTTTATTAGTGAAGCTTAGATAAAACTATGTTTGTATTAGAAGACCTAGTTTACATATTTGTCTG
GAGTCTCAAAATGGAACTGAATTCTGTCCATCTGATTGTGTCATACACAGAATATGCTCAA
TAAAAACCTTGGATAGTGATAAAATATATTCTGTCTTGAATTCCTTTTTTTCTTTAGGGTTC
TGTAAGCTATAGCTGGTGCTGTTATTCTGCTGGCTAAGAGACAATCTTGGAATTATAAAGACCT
CAGAAGAAATTGGTGAGTGTGTTCTAACAAAAG [G/T] TTAGAAAATCTGAAAAATGACACA
TTTCAGTATTTTATCTCTGCAAAGTAAATATCGATGCTTTGCCCCAAATGTGAT

Exon 13:

CCAGTTGTGTGATTTTTGTTTTGTTTTGTTTTAATGTTAGAAAACTTGCTAAAGAAGTAGG
TACTTCTTATGGCTGCTACTTCGTCCAGCATTTTCGGGGTAATATGCACCTTATTGGGAGC
CCAGCGCAAGAGGGTAAGTATTGAAAATATGGAGTGCTTTTGGGGATCTTGATTTAT

Exons 14 and 15:

TGTAAACGACGGCCAGTTGATTATGTCCAATTTTCTCTTCCTGGACATTTCTGTCTACCAA
ATTTGACCTTTTTCATATTTGAGATATTTCAAATTGATTGGTTTATATCATTCTAATCTGAAA
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TTTCTTGTACTTAGTTCACCTTTTATCACTCTTAAGTTATATGTTAACACCCGAGATTTATTC
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TAGGCATCaAGGCAGAAAAACAGGGTGCAAAATAGAGTTGTATAGCTTAGCTGAATATCAAG
GTGAATGCAGAGGTGTAGTGAGAGAAAAGGTTGGCTGTGACCAGATCAAAGAGGGCTTAGAA
GACCAGAATAAGAAGTCTCAATTTATTCCATAGGCTCTTGGAAGCTCTTGAGAGTTTCTGAG
TGGAGGATTGCCATTTTTCAGAGATGTTACTATGAAATAGATTTATAACATTAATTGCACTGG
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TAGATGGAGGAATGACCAGCAACAAAATTCCTTATGCAGCTACAAGCAGACATTCTGTATATA
CCAGTAGGTTAGTAAGTCTTCATTCCTTTAAACTCCCAGAGTAATGTTTCTTGTGGAATAAC
TAGTTCTTTGGG

Exon 16:

TGTAAACGACGGCCAGTTCCCAGAGTAATGTTTCTTGTGGAATAACTAGTTCTTTGGGCAT
ATGTAACCACAAAGATATTGATGGAACCTCTCTCTCCTCAGTGAAGCCCTCAATGCCCCGAAAC
CACTGCACTGGGTGCGGCTATGGCGGCAGGGGCTGCAGAAGGAGTCGGCGTATGGAGTCTCG
AACCCGAGGATTTGTCTGCCGTCACGATGGAGCGGTTTGAACCTCAGATTAATGCGGAGGGT
ACATTTAAAGAATGAAATGTTCAAGTATATACTGTGAAAACGACCTTAGTGCACGGGAGTTT
TGTTTTTCTGTTTAGTTAAAAGTTAAGGAACCAAGTAAAATAGTAAATGTTATCATTGCAGA
TTCGGCTGCCAAGCATATTGGGCTTTACTGAATAAATGTGAATGAGAGAAATCGTTGCTTAT
CAAAAGAACTTCTAAAATCACTTTTTTAAAATCATT

Exon 17:

TGTAAACGACGGCCAGTAGCCCTACTGCAGTTTAATGTGTCAATAATTTGTCAAGAATGTT
GAGTGATCATAAGTATGGTACTAAGAACATCTCAGCAAACCTACCTTTCGTTATGTGTTTTTT
CTACCTTCTAATTCTAGAAAGTGAAATTCGTTATTCTACATGGAAGAAAGCTGTGATGAAGT
CAATGGGTTGGGTTACAACCTCAATCTCCAGAAAGTGGTAAAAATGTTTTTGTATTATTATTGT
CACATTTTCTTAGTATATTAAATAGTTATTTAAGTATCTAGGCATTTACACATAGCCAGGCT
GCTCTGAAGAAAAGCATTATCATATGTCCAGAGATTCTGACATTTTGAAAACACTTTAAAGT
TCTAAACACAAAATGTAAATTATCAGGTGT

Figure 7C



Exon 18:

TGTAAAACGACGGCCAGTTGGTTTGGTTTGCTTGACTGGAATCTCTTCTGCTTGGATGACCA
CAGGTGACCCTAGTATCTTCTGTAGTCTGCCCTTGGGCTTTTTTATAGTGAGTAGCATGGTA
ATGTTAATCGGAGCAAGGTACATCTCAGGTAGTTACTCTTTAAATTAGACAACTCTATTAG
TTAGCTTTAATGTTTTTCGTGTATAACTTAGCAGAAATTTTTCAGTGTTTTTCATTCTTTCTG
TGTCTAGGAAGCTGGAAAATCAATTAAAGGTCTAATTAGTTAGACCAATTAATCTTTGGGGG
CAGTTAGAAGTAAGAACTGTGACTCTGCTTACCCTTTTTAAATTTTTAATGTGATGACTTCT
TTAAGAGGGACTACATTCTGCTGTCAGCTGCAGCAATAAGCAAAAGTGAAAATACTAATATT
TAAATGACAGGACTTTCAGACTGACTGCTGAAAGTTAAAGTATACTT

Exon 19:

AAAATTACTGGCTTAAATGGAAATGATGCTTCTTATTCTGTATGTTCCCATGAAAGTGAAAC
TTAAAAAATTCATGATTAGGGTTTCATGAAAAGGCCTTGTTTCTATGAAAATTGAGAC
AGGTTGCATCTCTCTAAGCTAAAAGATGGGCTATGTGTCTAGAGTCTTAGACTTCTAAAATG
CATGTGGTCACTATATGTAGGTTATCTCTTCGGTGACATACACTGCAATTTGAGAGGGCTGG
AAATTGTTTGCTTGGTAAACGATTAGCAACAGTGGCAATATTTGTTAATTTTGGAATTGGC
CCTGTTTGTGTCATTTTAATTGTGAGGCATGATTTAGAAATCATATGGACTTTCTAGCTTAA
TAAATGATTGAATCATCTGCATTGCTTTAACTCCTGAATTGTATGCATGTATTATTGACATA
TATGGTTTTTGTTCCTTCAGGTATTCATATAAACCTACCAACTCATGGATTCCCAAGA
TGTGAGCTTTTTACATAATGAAAGAACCAGCAATTCTGTCTCTTAATGCAATGACACTATT
CATAGACTTTGATTTTATTATAAGCCACTTGCTGCATGACCCTCCAAGTAGACCTGTGGCT
TAAAATAAAGAAAATGCAGCAAAAAGAATGCTATAGAAATATTTGGTGCTTTTTTTTTTTTT
TAAACATCCACAGTTAAGGTTGGGCCAGCTACCTTTGGGGCTGACCCCTCCATTGCCATAA
CATCCTGCTCCATTCCCTCTAAGATGTAGGAAGAATTCGGATCCTTACCATTGGAATCTTCC
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TTTGCCAAAACCTGAAATCCTTCAGATGTTTTCCATGGTCCCCTAATTATAATGACTTTCTG
TCTGGGTCTTATAGGAAAAGATACTTTCTTTTTCTTCCATCTTTCCTTTTTATATTTTTTA
CTTTGTATGTATAACATACATGCCTATATATTTTATACACTGAGGGAGCCCATTTATAAATA
AAGAGCACATTATATTAGAAGGTTCTAACAGGG

Figure 7D



Table 1. Characteristics of Carriers of the N288D GK Gene Mutation and of Their Unaffected Relatives

	Men			Women		
	N288D carriers	Unaffected relatives	p	N288D carriers	Unaffected relatives	p
N	18	18		14	14	
Age (years)	46.4±14.2	42.0±18.8	0.32	44.9±13.5	43.7±17.8	0.87
Uncorrected triglyceride (mmol/L) ⁽¹⁾	6.26±1.13	2.05±0.54	<0.0001	2.84±1.20	1.30±0.65	0.0002
Glycerol (mmol/L)	3.99±0.71	0.10±0.04	<0.0001	0.54±0.14	0.10±0.02	<0.0001
Corrected triglyceride (mmol/L) ⁽¹⁾	2.27±0.75	1.95±0.53	<0.0001	2.31±1.22	1.19±0.67	0.03
Free fatty acid (mmol/L)	0.77±0.22	0.57±0.25	0.01	1.29±0.35	0.76±0.17	0.0004
Fasting glucose (mmol/L)	5.2±0.74	4.8±0.31	0.13	5.0±0.7	4.6±0.3	0.10
2h glucose following OGTT (mmol/L)	7.9±3.1	5.8±1.6	0.02	7.0±6.1	5.0±2.1	0.04
Fasting insulin (mU/L) ⁽¹⁾	13.3±14.0	15.1±14.8	0.62	12.2±13.1	9.0±3.4	0.60
Waist girth (cm)	97.7±9.3	88.1±12.3	0.01	88.5±3.8	79.8±5.8	0.03
Body mass index (kg/m ²)	27.9±4.1	24.9±3.9	0.03	28.1±5.5	23.1±2.3	0.001
%Total body fat	27.1±7.2	22.9±7.6	0.01	46.8±8.1	33.9±11.3	0.001

(1) Geometric mean, p after log transformation.

Figure 8



Table 2. Fasting plasma glycerol concentration (mmol/L) in the initial cohort of 1056 individuals, by risk factor of glucose intolerance and diabetes mellitus

		No.	Glycerol geometric mean \pm SD	p
Gender	men	717	0.065 \pm 0.081	<0.0001
	women - premenopausal	137	0.071 \pm 0.093	
	- menopausal	202	0.099 \pm 0.085	
Age (Y)	<50	486	0.071 \pm 0.082	0.0015
	50 - 60	408	0.076 \pm 0.106	
	>60	165	0.083 \pm 0.053	
Fasting glucose (mmol/L)	< 5.2	449	0.068 \pm 0.080	<0.0001
	5.2 - 5.9	336	0.070 \pm 0.090	
	6.0 - 6.9	271	0.090 \pm 0.100	
Fasting insulin (UI)	<15	637	0.067 \pm 0.082	0.02
	\geq 15	419	0.086 \pm 0.101	
2 hours glucose (mmol/L)	<7.8	572	0.062 \pm 0.071	<0.0001
	7.8 - 11.0	283	0.081 \pm 0.101	
	\geq 11.1	201	0.102 \pm 0.110	
Triglyceride (mmol/L)	\leq 2.2	389	0.057 \pm 0.062	<0.0001
	>2.2	667	0.082 \pm 0.103	
Free fatty acid (mmol/L)	< 0.6	589	0.066 \pm 0.054	<0.0001
	\geq 0.6	467	0.111 \pm 0.112	
Body mass index (kg/m ²)	\leq 27	428	0.060 \pm 0.087	<0.0001
	>27	628	0.079 \pm 0.097	

p value from a one-way ANOVA

Figure 9

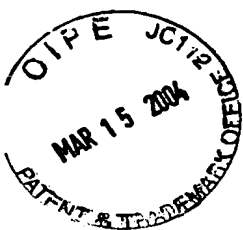


Table 3. Multivariate analysis of the relationships of fasting plasma glycerol concentration with impaired glucose tolerance (2h glucose 7.8-11.0 mmol/L following a 75 g oral load) before and after adjustment for covariates identified in

	Model 1	Model 2	Model 3	Model 4
Glycerol (log)				
β	1.75	1.62	1.46	0.77
Odds ratio	5.76	5.42	4.33	2.46
p	<0.0001	<0.0001	<0.0001	0.01
Triglyceride (log)				
β		0.54	0.35	0.12
Odds ratio		1.75	1.42	1.12
p		0.02	0.11	0.63
Body mass index (kg/m²)				
β			0.10	0.05
Odds ratio			1.10	1.05
p			<0.0001	0.01
Fasting insulin (log)				
β				0.57
Odds ratio				1.31
p				0.39
Fasting glucose (mmol/L)				
β				1.13
Odds ratio				2.65
p				<0.0001
Free fatty acid (log)				
β				1.62
Odds ratio				4.33
p				0.007

Odds ratios are expressed as the increase in the risk of 2h glucose 7.8-11.0 mmol/L following a 75 g oral load, associated with a 1-SD increase in the variables studied. β denotes the standardized estimate which is the parameter estimate of each variable in the multivariate logistic model. All models included age and gender as covariates. Otherwise, only the variables included in each model are shown. Subjects with severe hyperglycerolemia due to the N288D mutation in the